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BIOASSAY AND USE OF PITUITARY MATERIALS TO SPAWN WARM-WATER FISHES

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ABSTRACT

By bioassay, evaluation was made of the activity and relative potency of pituitaries collected throughout the year from various species of fish. Goldfish, zebra fish, and channel catfish were the chief test species; carp, green sunfish, largemouth bass, white crappie, and flathead catfish were used occasionally. Positive response was indicated by ovulation of eggs or by increase in seminal plasma.

In experiments to determine the species from which pituitaries can be used to spawn various species (phylogenetic specificity), 29 interspecific and intergeneric injections and 25 interfamilial injections were successful; only 7 heteroplastic injections failed to induce ovulation or exhibited low activity. Of the 7 failures, 4 involved river carpsucker and fresh-water drum pituitaries at regular dosage levels; and the other 3 were unsuccessful attempts to spawn carp with other than homoplastic pituitaries. Specificity appears so slight that it has no practical importance in fish-cultural techniques.

Activity of pituitaries collected in various months did not vary as much as the individual variations in physical condition of the test fish. The data indicate that all the pituitaries are active in the months in which collections were made. Activity in relation to size, sex, and degree of gonadal development of the donor fish does not appear as important as implied by some previous reports in the literature.

No detrimental effects of pituitary injections have been detected in incubating and hatching the eggs, or in rearing the fingerlings, from over 200 channel catfish spawns and thousands of goldfish spawns.

BIOASSAY AND USE OF PITUITARY MATERIALS TO SPAWN WARM-WATER FISHES

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Use of pituitary materials to induce fish to spawn has many advantages in the culture of sport, food, bait, and tropical fishes. Since fully ripe females of most species respond to pituitary injections by spawning within 12 to 20 hours, it is possible to predict rather accurately when a spawn will occur. This predictability allows the fish-culturist to plan his work according to a rigid schedule, since the injections, to some degree, bypass the variables in the environment, such as temperature, light, and rain. Since both males and females respond positively to pituitary injections, most species can be hand-stripped, a practice which offers additional advantages. Culture ponds can be stocked with eggs and fry of uniform age and size. Since the brood stock are not in the pond with the offspring, the possibility of transmission of disease from brood stock to offspring is reduced, and predation by the parent is eliminated. Hybrids can also be produced by hand-stripping whenever hybridization is desirable and feasible. Wild fish that have not yet been domesticated may be spawned more easily by the pituitary method. Frequently more uniform spawning has been achieved by the injection of pituitary materials into ripe fish which, because of some environmental or physiological condition, would fail to spawn without such treatment.

South American and Russian fish-culturists have applied the pituitary method to the large-scale spawning of fish of economic importance. In the United States such work has been limited for the most part to small experimental lots of fish. This is pointed out by Pickford and Atz (1957), who reviewed and discussed all the available literature on the use of pituitaries. A study of their book, indispensable to anyone interested in fish gonadotropins and their use in fish culture, reveals: (1) Fish pituitaries contain the hormones to precipitate spawning in ripe fish. (2) There is little if any specificity in the hormones of fishes; that is, the pituitaries of one family or species are usually active in unrelated species. (3) There are a number of examples in which gonadotropic materials from other vertebrate classes were found to be active in fish. (4) Out-of-season spawning has been induced by the administration of gonadotropins. (5) Fresh, acetone-dried, or frozen pituitaries, as well as glycerine extracts, saline suspensions, and water extracts of pituitaries have similar activity. (6) Under most experimental conditions the injection of pituitaries does not decrease fertility. (7) Increased or de-

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creased exposure (depending on species of fish involved) to light may have the same effect on sexual development and spawning as pituitary injections. (8) The lack of response in some experiments with mammalian gonadotropins may be due to the fact that the dosage was too low (Kawamura and Otsuka, 1950, used quite high levels to achieve positive results). Also, fish may require a ratio of components different from that contained in some mammalian materials; and there may be a quantitative difference in the ratio of hormones rather than a qualitative difference in the hormones themselves. (9) There is little or no qualitative difference in the pituitaries of the male and female donor fish, at least from a practical viewpoint. (10) There may be a "seasonal" difference in the biological effect produced by pituitaries taken from fish prior to spawning and those taken immediately after spawning.

Much more must be learned before the qualitative and quantitative differences in fish pituitaries at various times in the year are known. The responses to these pituitaries of fish of different physiological condition, age, and development must also be determined. At present, good bioassay methods for fish gonadotropins are unknown. Witschi (1940) demonstrated that the LH component of vertebrate pituitaries could be assayed relatively by injecting the material into weaver finches. The method for the bioassay of FSH in fish pituitary materials involves the cornification of the mouse vagina, but these methods have been questioned by some workers. Until good methods are devised, rather crude bioassay methods of whole pituitaries and unpurified extracts must be used.

BIOASSAY OF PITUITARY MATERIALS

Collection and preparation of pituitaries

There are many potential sources of fish pituitaries. When only a few pituitaries are needed it is usually practical to sacrifice the culls from the brood stock which is to be spawned. Our experience has indicated that these culls have active pituitaries. Also, they are usually similar in size to fish to be spawned and their pituitaries can be injected without weighing or diluting. At some fish hatcheries it is advantageous to have a stock of carp or goldfish to provide a few pituitaries when needed. Often wholesale fish markets may handle fish from which pituitaries can be obtained, provided that the fish are strictly fresh or were frozen when fresh. Pituitaries (mostly from buffalo, *Ictiobus*) that were frozen in the head of fish for a few days up to at least 1 year were effective in spawning goldfish, but these were not as uniform or as potent in action as fresh material. An excellent source of a large number of pituitaries is from the rough fish caught by commercial fishermen or State fish and game department employees when they use traps, nets, or rotenone. Pituitaries which are allowed to remain at high temperatures after the death of the fish probably lose their gonadotropic activity because of enzymatic action.

Our method of collecting the pituitaries was to cut off the heads of the fish with a cleaver, remove the top of the cranium of each immediately above the eyes, and lift out the brain with forceps. In some species the pituitary remained attached to the ventral part of the brain; in other species it was left in the myodom in the floor of the brain case. The pituitary was removed with the aid of forceps. In large-scale collections, 9 men could collect about 1,000 pituitaries per hour. There are some species of fish, such as gar (*Lepisosteus*) and gizzard shad (*Dorosoma*), whose pituitaries are so difficult to

remove that collection is hardly worthwhile. The pituitaries of the gar had to be exposed by use of a bandsaw because of the ganoid scales, and the pituitaries of the shad are extremely delicate.

The donor fish (those from which the pituitaries were removed) were always sorted according to species. Unless they were to be used for special experiments, no attention was given to sex, maturity, or size, although fish of sufficient size were chosen to justify the effort of collecting the pituitaries. In most cases, we assumed that the smaller fish in our collection would probably reproduce during the next spawning season.

Since the pituitary gonadotropins are proteins subject to enzymatic deterioration, it is logical to assume that pituitaries frozen or placed in acetone promptly after removal will produce better gonadotropic activity. Some pituitaries were placed in vials in the field and immediately frozen in insulated cans of dry ice. These were later freeze-dried in the laboratory. If acetone was used, the pituitaries were dehydrated in four changes of cold acetone 12 hours apart. Drying-vials should be kept full of acetone so that moisture from the air will not condense on the inside walls of the vials and dilute the acetone. For the same reason, the acetone of the last change should be allowed to warm to room temperature before it is removed and the pituitaries allowed to air-dry.

Pituitaries preserved by either method should be stored in a dry atmosphere, preferably in a dessicator charged with calcium chloride or other drying agent. If the pituitaries are to be used promptly or if the humidity is low, tightly stoppered vials can serve as temporary containers. We have found that pituitaries treated in this manner are potent after 2 years (our longest data period), but Hasler, Meyer, and Wisby (1950) reported that acetone-dried carp pituitaries stored and preserved in this way were active after 10 years.

Dry pituitaries were finely ground and injected into the body cavity as a suspension in distilled water (other diluents, such as physiological saline or isotonic salt solution, theoretically should be less harmful to the tissues, but distilled water combined with dried pituitaries gave acceptable results and appeared to be relatively harmless to the fish). Fresh pituitaries were injected by placing the gland in the socket of the needle and forcing it through the needle stem with distilled water. The site of injection was usually at the axil of the pelvic fin. Intramuscular injections were also given, but the body-cavity injection was more satisfactory. Intramuscular injections are more convenient when the fish are so large that it is cumbersome to remove them from the tank, but such injections are more likely to produce lesions that damage the fish. When injected intraperitoneally the diluent volume can be larger and less critical, and injections can be more easily and quickly administered.

Other carriers as well as distilled water were tested. They were mixed with whole, ground, acetone-dried pituitaries and injected at "full-dose" (0.7 milligram) and "half-dose" (0.4 milligram) levels. One of these, cholesterol, was mixed with ground pituitaries to give a ratio of 300 : 7 at the "full-dose" level and 300 : 4 at the "half-dose" level. This mixture was compacted into cylindrical pellets which were injected with a caponizing needle into the body cavity of the fish. Similar dosages of pituitary materials containing 1 milliliter of distilled water or of sesame oil were injected into the body cavities of other fish. In this experiment female goldfish were divided into groups of four and injected as listed in table 1.

The cholesterol and sesame oil were used to reduce the rate of absorption and to make absorption more uniform over a longer period of time. It appeared that the absorption rates were changed, but the

change was not advantageous to our present problem of spawning ripe fish.

Pickford and Atz (1957) reviewed the use of glycerine extracts of pituitaries and pointed out the reported possible advantages of greater gonadotropin recovery, slower absorption, and ease of storage in sealed vials at room temperature. We wish to add that injections of glycerine extracts are easier to administer than aqueous suspensions of pituitaries, but the preparation of these extracts is tedious. De Menezes, Fontenele, and Comacho (1945) injected small volumes (0.5) milliliter) of glycerine extract which contained as high as 66 percent glycerine. The use of this high percentage of glycerine theoretically is biologically inconsistent with the osmotic properties of the body cavity. Since the volume of aqueous suspensions most often used in our experiments was about 1 milliliter, we injected goldfish (*Carassius auratus*) with this volume of water with the following percentages of glycerine: 1, 2.5, 5, 10, 20, 30, 40, 50, 60, and 70. Each injection contained 10,000 units of penicillin, but contained no gonadotropin. All fish that received injections of 10 percent or less glycerine suffered no harmful effects after 3 days at 60° F., while fish that received injections containing more than 10 percent glycerine showed excessive fluid in the body cavity, peritoneal inflammation, and enlarged hemorrhagic areas at the point of injection. On this basis, we decided to keep the glycerine content below 10 percent whenever possible, even though another experiment (table 2) indicated that small volumes of higher percentages of glycerine were suitable.

In a limited experiment with goldfish, injections of equivalent amounts of acetone-dried carp pituitary as aqueous suspensions and as glycerine extract (table 2) showed little difference in effectiveness.

Another experiment with channel catfish (*Ictalurus punctatus*) indicated (so far as the effectiveness or the quantity of the pituitary used for injection is concerned) that glycerine extract was less effective than aqueous suspensions since only 1 female in 19 spawned. However, we feel that such a conclusion is unwarranted on the basis of this one experiment. The fish in the experiment had been starved for oxygen while being transported 2 days before use and had been both temperature-shocked and briefly oxygen-starved on the day the experiment began. After the end of the glycerine-extract experiments, nine fish which had received the glycerine injections were spawned by the injection of other gonadotropins (fish pituitary or human chorionic gonadotropin), but one might postulate that they had recovered from the shock, since the time of final spawning was several days later.

Crystalline penicillin at the rate of 10,000 units per injection was found to be an inexpensive and effective measure to combat inflammatory lesions in the body cavity due to irritation from the foreign proteins.

TABLE 1.—*Female goldfish* (Carassius auratus) *ovulated with dried pituitary in various carriers*

Carrier	Dose	Number injected	Number ovulated
Distilled water	Full	4	4
Do	Half	4	4
Cholesterol	Full	4	4
Do	Half	4	4
Sesame oil	Full	4	1
Do	Half	4	1

TABLE 2.—*Comparison of effect of injections of aqueous suspensions of pituitaries and glycerine extract in female goldfish* (Carassius auratus) *at water temperature of 60° F.*

Dosage level (equivalent acetone-dried carp pituitaries)	Volume of injections (milliliters)	Ratio of ovulating females to number injected		
		Aqueous suspension	75-percent glycerine extract	84-percent glycerine extract
0.0 milligrams [1]	1.0	0:3	0:3	0:3
0.2 milligrams	0.1	1:3	3:3	1:3
0.5 milligrams	0.25	3:3	3:3	2:3
1.0 milligrams	0.5	3:3	2:3	0:3
1.5 milligrams	0.75	2:3	2:3	1:3
3.0 milligrams	1.5	3:3	[2] 1:1	0:3

[1] Control. [2] 2 died.

Although thousands of goldfish and the majority of experimental fish used in these studies usually spawned or ovulated after a single injection of pituitary materials without penicillin, it appeared later that in such fishes as the channel and flathead catfish penicillin acted to minimize the inflammation which resulted from the multiple injections of pituitary materials necessary to spawn these species. Our opinion was based on the difference between results we obtained before the routine use of penicillin and those we obtained at a later time with its use. No good, controlled experiments were conducted. In the original experiment that heralded this practice, goldfish received from 5,000 to 100,000 units of crystalline penicillin per dose for five injections over a 9-day period. Ground pituitaries were added to the injection on the 9th day, and the fish spawned on the 10th day in the manner expected. Now we recommend the injection of penicillin for all species as a precautionary measure. The penicillin is dissolved in distilled water, which is then added to the ground pituitaries. Dr. S. F. Snieszko recently suggested that chloramphenicol might be more effective for this purpose since it might also prevent gram negative bacterial infections, whereas penicillin is active against gram positive bacteria which do not often infect fish. The suggested dosage was 5 milligrams per 100 grams of body weight of the recipient.

Determination of active pituitaries

In our studies of inducing fish to spawn, it was desirable to learn the activity and relative potency of pituitaries collected throughout the year from various species of fish and from various types of individuals within the species, especially in regard to sex, maturity, and size. For these tests, bioassay was the only available means of evaluation. A test fish was needed, and the factors that determined its selection were as follows: (1) It should be readily available and easily kept in the laboratory. (2) It should respond predictably and uniformly to the injection of pituitary materials. (3) The nature of its response should lend itself to an objective rather than a subjective evaluation; for example, the ovulation and extrusion of eggs was used rather than visible increases in the weight of the gonad of the living fish. (4) Preferably, it should be an oviparous species of fish which spawns intermittently throughout the year.

There are several fishes that fulfill these requirements, and the selection of a test fish may vary with the prevailing circumstances. We have used female goldfish extensively during the normal spawning season (March-June) since they are more responsive at this time, and tests can be conducted with greater ease. Zebra fish (*Brachydanio rerio*), despite their small size, are good test fish because gravid females can be obtained anytime during the year; and since they respond to low dosages their use results in economy of pituitary materials. When female fish are employed, a positive test is obtained when the injection of pituitary material induces ovulation or precipitation of the spawning act.

We have also used male green sunfish (*Lepomis cyanellus*), carp (*Cyprinus carpio*), and goldfish (*Carassius auratus*) when ripe female fish were not available. Generally, these tests were not during the spawning season. Milt can be readily stripped from most males injected with pituitary material. The amount of the spermatic fluid will increase and will be less viscous. The response is particularly evident in out-of-season fish of species in which spermatogenesis is well advanced by late summer or early fall. For example, in midwinter the semen of carp (in which the spermatozoa are normally closely packed in the viscous seminal plasma) becomes very fluid and will actually drain

from the fish about 20 hours after injection (Sneed and Clemens, 1956). A male which yielded about 0.1 milliliter of milt before injection produced about 10 milliliters after receiving a pituitary injection.

A similar response has been observed in the white crappie (*Pomoxis annularis*), white bass (*Roccus chrysops*), largemouth bass (*Micropterus salmoides*), goldfish (*Carassius auratus*), and golden shiner (*Notemigonus crysoleucos*). The increase of the fluidity of the milt after injection of pituitary has been observed in several other species of fish (Ihering and de Azevedo, 1934). Ihering (1935) used the length of time that the spermatozoa remained active when put into water as a measure of their maturity, and reported that pituitary injections into the donor male increased longevity of the sperm. We have not studied this effect, although we have observed changes in the osmotic pressure of the seminal plasma in carp which corresponded to the changes of fluidity (Sneed and Clemens, 1956). We feel that here is a potential method of measuring the relative activity of gonadotropins—the strength of the gonadotropin being equal to the difference in osmotic pressure of the seminal plasma before and after injection. For instance, carp sperm was immobilized with 85-percent Ringer solution (15 percent less water) while a 75-percent solution (more concentrated salts and higher osmotic pressure) was necessary for the inactivation of the sperm 15 hours after the fish had received one carp pituitary.

As the spawning season approached and the fluidity of the milt normally increased, the osmotic pressure of the seminal plasma increased—at least it required a solution of a higher salt concentration than it did earlier to maintain the sperm in an immotile state. Male zebra fish have not been used in this way, because the increase of milt is difficult to observe with the unaided eye. The morphology of the testes of channel catfish or of other ictalurids does not permit the use of hand-stripping methods; therefore the male of these species is not a potential test fish.

When the kind of fish and the type of test to be conducted has been determined, the individuals for the experiment must be selected. The selection of fish is discussed in the second part of this report.

Determination of dosages

When the experimental fish have been properly selected, suitable spawning facilities provided, and pituitaries of known activity secured, the determination of dosages may require a great deal of experimentation if the recipient species has never been spawned before. The major problems involved are: (1) How many milligrams of acetone-dried pituitary are required to precipitate spawning? (2) How many injections are required? (3) What is the optimum time interval between each injection?

If the species has not been spawned before, lots of three or more fish should be injected each 24 hours with a fairly wide range of pituitary dosages until spawning occurs. In our experiences only the females need to be injected during the spawning season. If there is any reason to doubt whether the male will spawn, perhaps he should be injected; however, the necessity for such injections has not been positively established for any species with which we have worked.

If the species has been spawned previously, the above questions have been partially answered. It is then a matter of matching the dose to the physiological condition of the recipients by administering doses in a graded series from above to below those recommended in this paper or those in other publications for other species of fish.

These pilot experiments will indicate the lowest dosage that will spawn the maximum number of fish with the minimum

number of injections. Such a dosage should be determined for each lot of fish. It usually saves time and labor to administer doses slightly above the minimum required for spawning, rather than to risk failure with subminimal doses. Once a threshold dosage has been reached, no matter how much the dosage is increased, the results do not seem to change (table 3).

Phylogenetic specificity

One of the first questions to arise in the artificial spawning of any species of fish is what kind of pituitaries should be used for injection. In our experiments the goldfish has responded to injections of pituitaries from the following 15 species of fish representing seven families: Goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), bigmouth buffalo (*Ictiobus cyprinellus*), smallmouth buffalo (*I. bubalus*), black buffalo (*I. niger*), blue sucker (*Cycleptus elongatus*), gizzard shad (*Dorosoma cepedianum*), channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), flathead catfish (*Pylodictis olivaris*), black bullhead (*Ictalurus melas*), spotted gar (*Lepisosteus productus*), and extracted chum salmon (*Oncorhynchus keta*) pituitary (table 4). Low activity was obtained from the pituitaries of freshwater drum (*Aplodinotus grunniens*) and river carpsucker (*Carpiodes carpio*).

A series of experiments was conducted to determine whether such factors as the method of preservation of the pituitaries, the place of collection, the size of the donor fish, the amount of light to which the fish had been exposed, inhibiting materials in the pituitary, and the dosage level are responsible for the low activity of carpsucker pituitaries. Acetone-dried and lypholyzed pituitaries, pituitaries from fish from Lake Texoma and Fort Gibson Reservoir, pituitaries collected from December to April, and pituitaries from fish kept in the light and in the dark for a period of 3 weeks, all gave negative

TABLE 3.—*Response of different lots of goldfish* (Carassius auratus) *to pituitary dosages*

Dosage	Number injected	Number ovulated
Buffalo (*Ictiobus* spp.) pituitaries, Feb. 19, 1957, at 72° F.:		
0	2	0
⅛ pituitary	4	1
½ pituitary	4	3
1 pituitary	4	3
Buffalo (*Ictiobus* spp.) pituitaries, March 6, 1957, at 72° F.:		
0	4	0
0.2 milligram	4	1
0.3 milligram	4	2
0.9 milligram	3	0
1.7 milligrams	4	3
3.3 milligrams	4	3
4.9 milligrams	4	3
6.3 milligrams	4	3
Carp (*Cyprinus carpio*) pituitaries, March 18, 1957, at 72° F.:		
0	4	0
0.5 milligram	16	4
1.0 milligram	16	9
1.5 milligrams	32	21
Carp (*Cyprinus carpio*) pituitaries, April 7, 1958, at 60° to 62° F.:		
0	3	0
0.2 milligram	3	1
0.5 milligram	3	3
1.0 milligram	3	3
1.5 milligrams	3	3
3.0 milligrams	3	3

results when injected into goldfish that were known to be responsive to injections of a similar size of pituitaries from other kinds of fish.

Carpsucker pituitaries are relatively small. The average weight of the thousands we collected and freeze-dried was 1 milligram, and these were collected from fish 10 to 15 inches long. When large dosages of either aqueous suspensions or acid-acetone extracts were injected, some activity was observed. Only 25 percent successful ovulations were produced in one experiment in which 3, 6, and 9 milligrams (4 to 10 glands) of carpsucker pituitary were injected in each fish of three groups of four goldfish. Another experiment in which acetone-dried pituitaries from carpsuckers were injected whole and without water by use of a pellet injector indicated that extremely high dosages were required to ovulate female goldfish. Four females received injections at each dosage level. No female ovulated at dosages of 1 milligram (1 gland); one ovulated with a dosage of 5.1 milligrams (5 glands), one with 10.2 milligrams (10 glands), two with 23

TABLE 4.—*Responses of 12 species of fish to pituitaries from donors of various taxonomic relationships*

Recipient species	Donor species	Intraspecific	Interspecific	Intrageneric	Intergeneric	Intrafamilial	Interfamilial	Intraordinal	Interordinal	Remarks
Goldfish (*Carassius auratus*)	Goldfish (*Carassius auratus*)	X		X		X		X		
Do	Carp (*Cyprinus carpio*)		X		X	X		X		Responded to both acetone-dried pituitaries and acid-acetone extract (Robertson and Rinfret, 1957).
Do	Bigmouth buffalo (*Ictiobus cyprinellus*)		X		X		X	X		
Do	Spotted gar (*Lepisosteus productus*)		X		X		X		X	
Do	Flathead catfish (*Pylodictis olivaris*)		X		X		X	X		
Do	Channel catfish (*Ictalurus punctatus*)		X		X		X	X		
Do	Blue sucker (*Cycleptus elongatus*).		X		X		X	X		
Do	Gizzard shad (*Dorosoma cepedianum*).		X		X		X		X	
Do	Black bullhead (*Ictalurus melas*).		X		X		X	X		
Do	Blue catfish (*Ictalurus furcatus*).		X		X		X	X		
Do	Smallmouth buffalo (*Ictiobus bubalus*).		X		X		X	X		
Do	Black buffalo (*Ictiobus niger*)		X		X		X	X		
Do	Chum salmon (*Oncorhynchus keta*).		X		X		X		X	Cholesterol pellets of Robertson-Rinfret extractions; furnished by Roger Burrows.
Do	Freshwater drum (*Aplodinotus grunniens*).									May have been too low in activity for response at dosages administered.
Do	River carpsucker (*Carpiodes carpio*).	X								Some activity at higher dosage levels; positive with Robertson-Rinfret extractions.
Carp (*Cyprinus carpio*)	Carp (*Cyprinus carpio*)	X		X		X				Acetone-dried pituitaries, extracts, and fresh pituitaries.
Do	Buffalo (*Ictiobus* sp.)									
Do	Channel catfish (Ictalurus *punctatus*).									
Do	Goldfish (*Carassius auratus*)									
Golden shiner (*Notemigonus crysoleucas*).	Carp (*Cyprinus carpio*)		X		X	X		X		
White crappie (*Pomoxis annularis*).	Carp (*Cyprinus carpio*)		X		X		X		X	
Do	Chum salmon (*Oncorhynchus keta*).		X		X		X		X	Cholesterol pellets containing Robertson-Rinfret extract.
White bass (*Roccus chrysops*).	Carp (*Cyprinus carpio*)		X		X		X		X	
Zebra fish (*Brachydanio rerio*).	Buffalo (*Ictiobus* sp.)		X		X		X			
Do	River carpsucker (*Carpiodes carpio*).		X		X		X			Dosage level high.
Do	Carp (*Cyprinus carpio*)		X		X		X			
Channel catfish (*Ictalurus punctatus*).	Channel catfish (*Ictalurus punctatus*).			X		X		X		
Do	Carp (*Cyprinus carpio*)		X		X		X	X		
Do	Freshwater drum (*Aplodinotus grunniens*).									
Do	River carpsucker (*Carpiodes carpio*).									
Do	Buffalo (*Ictiobus* sp.)		X		X		X	X		
Do	Gar (*Lepisosteus* sp.)		X		X		X		X	
Do	Flathead catfish (*Pylodictis olivaris*).		X		X	X		X		
Rock bass (*Ambloplites rupestris*).	Buffalo (*Icitiobus* sp.)		X		X		X		X	
Emerald shiner (*Notropis atherinoides*).	Carp (*Cyprinus carpio*)		X		X	X		X		
Mimic shiner (*Notropis volucellus*).	Buffalo (*Ictiobus* sp.)		X		X		X	X		
Redfin shiner (*Notropis umbratilis*).	Buffalo (*Ictiobus* sp.)		X		X		X	X		
Flathead catfish (*Pylodictis olivaris*).	Flathead catfish (*Pylodictis olivaris*).	X		X		X		X		
Do	Buffalo (*Ictiobus* sp.)		X		X		X	X		
Do	Carp (*Cyprinus carpio*)		X		X		X	X		

milligrams (20 glands), four with 36.2 to 46.6 milligrams (40 glands), two with 54.7 to 70.2 milligrams (60 glands), and three with 78.2 to 98.1 milligrams (80 glands).

In almost all of the negative instances when 9 or more milligrams were injected, blood exuded from the oviduct when hand-stripping was attempted. This response suggested an overdose for those fish; but since the eggs in the fish that did ovulate appeared normal and no blood was seen, it cannot be implied that the amount of pituitary injected in the instances of negative response constituted a pharmacological overdose, but rather a physiological one.

Several species of fish were spawned with whatever kind of pituitaries were available (table 4). Phylogenetic specificity does not appear to be significant in fish-cultural practices (Pickford and Atz, 1957), and our experience bears out this viewpoint. Twenty-nine interspecific and intergeneric injections and 25 interfamilial injections were successful. Only seven heteroplastic injections failed to induce ovulation or exhibited low activity. Four of the seven failures involved the use of carpsucker and drum pituitaries at regular dosage levels, while the other three were unsuccessful attempts to spawn carp with heteroplastic pituitaries. However, carp were successfully spawned by other workers who used heteroplastic implants (see Pickford and Atz, 1957).

Kazanskii (reviewed by Pickford and Atz, 1957) implied that the carp, *Cyprinus carpio*, was in effect a universal donor. In our work, carp pituitaries successfully spawned nine species of fish. There may be other species of donor fish with equally active pituitaries, for instance, buffalo. However, carp pituitaries are usually more available, and their more extensive use has led workers to regard them as superior. Pickford and Atz (1957) state that the work with fish provides clear indications, but no proof, of specificity. Our present studies indicate that there was little specificity between families and lower taxonomic categories. At least, the differences were so slight that they have no practical importance in fish-cultural techniques. However, an explanation is certainly needed for the lower activity of carpsucker and drum pituitaries. It may be that the carpsucker pituitaries were taken largely from immature fish. Drum pituitaries have not been tried in enough experiments to make a definite conclusion, although we have used them a dozen times or more.

Gonadotropic activity in relation to season

It is important to be relatively certain that the pituitaries collected for the purpose of injection are active, as injections with inactive materials would waste valuable time and perhaps fish. We attempted to devise experiments that would determine the seasonal activity of the pituitaries of several species of fishes, but our supply of pituitaries was not always sufficiently large to provide materials for the basic experiments of inducing spawning in channel catfish as well as for comparative tests of materials collected in different months. The limited significant data on seasonal activity that we obtained are presented in table 5. These data represent successful attempts to induce ovulation or spawning of several species and indicate that the pituitaries were active in each of the months listed. We have no data which indicate that the pituitaries of any species are inactive during any month in which we made collections. The pituitaries of the carp proved to be active in at least 9 months of the year (we did not test carp pituitaries collected in May, August, or September); those of buffalo (*Ictiobus* spp.) were active in 8 months, including May and August (table 5). No seasonal difference in pituitary activity was discernible from the injections which

TABLE 5.—*Activity of pituitaries collected in different months from eight donor species*

[X indicates that spawning was induced when the pituitaries were injected in any of several test species]

Species	Donor fish collected in—											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Carp (*Cyprinus carpio*)	X	X	X	X		X	X			X	X	X
Buffalo (*Ictiobus* spp.)	X	X			X		X	X		X	X	X
River carpsucker [1] (*Carpiodes carpio*)	X	X	X	X								X
Flathead catfish (*Pylodictis olivaris*)	X					X	X					X
Gar (*Lepisosteus* spp.)							X				X	
Goldfish (*Carassius auratus*)			X	X								
Channel catfish (*Ictalurus punctatus*)		X										
Blue sucker (*Cycleptus elongatus*)						X						

[1] Pituitaries of this species only slightly active.

we made to induce spawning. This fact does not necessarily indicate that seasonal differences do not exist. We believe that our experiments were not sufficiently sensitive to indicate variability in pituitary activity from month to month that could not be explained equally well on the basis of inconsistencies in the bioassay method and/or the individual physiological variability of the recipient species.

On the basis of the information now available on fish gonadotropins, the biological assumption may be made that there is a seasonal difference in the amount of hormones in the blood or in the amount utilized by the target organ. This assumption does not necessarily include a difference in the concentration of gonadotropins in the pituitary itself. Gerbil'skii (reviewed by Pickford and Atz, 1957) generally concluded "that the presence—and therefore presumably the production—of fish gonadotropin is low only for a period of varying length following spawning." He believed that the gonadotropic content of pituitaries from fish that spawned in the spring remained relatively low into October. However, his data are not conclusive, and as we have pointed out many times, the bioassay method is inconsistent, and often the recipients may vary physiologically more than the pituitaries vary in gonadotropic content. Unlike Brazilian practices of collecting glands from only ripe or ripening fish, we recommend the collection of pituitaries from mature fish during any month of the year at the convenience of the worker.

Gonadotropic activity in relation to sex of donor

Pickford and Atz (1957) reviewed the literature on differences in gonadotropic content of the pituitary of males and females. They stated that although fish-culturists use the glands of either sex indiscriminately, they can no longer assume that the gonadotropic content of male and female pituitaries is the same. They cited the work of Barannikova (1949) which showed that pituitaries of male sevringa (*Acipenser stellatus*) when tested in *Misgurnus fossilis* were at times only half as potent as those from females. With the donors and recipients used in our work and in large-scale operations in fish culture, we have found nothing to be gained from separating the pituitaries of the two sexes. In practical application, when fully ripe fish are used, selected dosage levels are usually more than minimal, since there is no apparent harm to the fish or the spawn with moderate overdoses. Slight overdoses tend to offset individual differences in both the pituitaries and the recipient fish. Such differences in activity due to sex or other variables are further minimized by grinding together pituitaries of various collections and injecting known quantities of the ground mixture.

TABLE 6.—*Response of female goldfish* (Carassius auratus) *injected with whole or with parts of transected buffalo* (Ictiobus *sp.*) *pituitaries*

Kind of pituitary injection	Weight of pituitary injected (milligrams)	Number of injected fish	Number of ovulating fish	Part of pituitary injected	Weight of pituitary (milligrams) before transection	Number of injected fish	Number of ovulating fish
Whole	6.3	4	2	Anterior	1.6	4	3
Whole	4.9	4	3	Posterior		4	0
Whole	3.3	4	3	Anterior	1.5	4	3
Whole	1.7	4	3	Posterior		4	0
Whole	0.8	4	0	Anterior	3.1	4	[1] 2
Whole	0.4	4	2	Posterior		4	1
Whole	0.3	4	1				
Control	0	4	0				

[1] The negative fish in this group were considered poorly developed at the time the experiment began.

Site of gonadotropic activity in the pituitary

In order to determine which part of the pituitary contained the active gonadotropins, an experiment was designed which (1) determined a dosage of whole buffalo pituitary (about 1.7 milligrams per fish, table 6) necessary to induce ovulation in at least three of four goldfish (this information was necessary to assure the use of pituitaries of adequate size in the second part of the experiment) and (2) tested the gonadotropic activity of the anterior and posterior parts of three pituitaries of approximately similar size or larger by injecting each part into separate groups of four goldfish (table 6). Posterior lobe here refers to the meta-adenohypophysis, as suggested by Pickford and Atz (1957); anterior lobe refers to the remainder of the pituitary.

In evaluating the response of the injected fish approximately 15 hours after injection, only those fish that readily responded to hand-stripping were counted as positive. About 69 percent of the females responded to injections of ground whole pituitaries when the dosage was 1.7 milligrams or more; 71 percent of the females responded to injections of the anterior lobes of pituitaries weighing 1.5 milligrams or more; no ovulation occurred in the eight fish which received the posterior part of the pituitary. These results of the effect of buffalo pituitaries on goldfish confirmed reports that the source of gonadotropic activity is found in the anterior lobes of the pituitary, and that the posterior lobe contains no active gonadotropins. When the dosage levels were doubled, results were as expected—positive results with the anterior portion and some activity with the posterior portion. Eggs were easily hand-stripped from one female injected with posterior lobe. These results were probably the outcome of the method of dissection: pieces of the anterior lobe were left on the posterior lobe so that enough anterior lobe material had accumulated to be active in the higher injection. Other workers (Kazanskii and Persov, Barannikova, see Pickford and Atz, 1957, for review) transected pituitaries and demonstrated that the anterior portion is the site of gonadotropic activity.

Relation between pituitary size and gonadotropic activity

The gonadotropic activity of different sizes of pituitaries (acetone dried) was indicated by the percentage of induced ovulation of goldfish injected with standard amounts (1.5, 1.0, and 0.5 mg.) of pituitaries of varying sizes, e.g., eight groups of carp pituitaries varying in size from one to eight per 10 milligrams (combined weight) and five groups of buffalo

TABLE 7.—*Gonadotropic activity of various sizes of carp and buffalo pituitaries as indicated by induced ovulation of injected goldfish*

[Data based on fish rated "good"]

CARP PITUITARIES

Number of carp pituitaries per 10 milligrams	Dosage 1.5 milligrams		Dosage 1.0 milligrams		Dosage 0.5 milligrams	
	Number injected	Percent of ovulation	Number injected	Percent of ovulation	Number injected	Percent of ovulation
1	2	100	3	66.6	2	50
2	4	100				
3	3	100	2	0	1	100
4	4	100				
5	2	100				
6	1	100	2	100	1	0
7	3	100				
8	1	0	3	100	1	0

BUFFALO PITUITARIES

Number of buffalo pituitaries per 6.5 milligrams	Dosage 1.5 milligrams		Dosage 1.1 milligrams		Dosage 0.75 milligrams		Dosage 0.37 milligrams	
	Number injected	Percent of ovulation	Number injected	Percent of ovulation	Number injected	Percent of ovulation	Number injected	Percent of ovulation
1	2	50	1	100	2	50	2	0
2	3	66.6	1	0	4	100	2	100
3	1	100	4	75	2	50	1	100
5	2	50						
6	3	100						

pituitaries varying from one to six per 6.5 milligrams (table 7).

The recipient fish for these experiments were not selected, but were arbitrarily assigned to aquariums and rated as good, fair, and poor; thus the results reflect the condition of these fish as well as the activity of the pituitaries of the donors. The ratings were made before the experiment, and we used only the data obtained from fish rated "good".

A fish rated "good" was one we believed to have a good possibility of ovulating or spawning, doubtful fish were rated "fair," and "poor" fish in all probability would not ovulate or spawn. At the dosage of 1.5 milligrams per fish all of these estimates of condition were above 80 percent correct—94.2 percent correct for the "good" ratings, 80 percent for "fair," and 100 percent for "poor."

If only the fish rated "good" were considered at the standard dosage level (1.5 milligrams) for any size of pituitary, then the size differences were not significant provided the total weight of pituitary was the same (table 7). Therefore, the activity per unit weight did not vary with the size of carp pituitaries weighing from 1.0 to 10 milligrams, the gonadotropic activity being directly proportional to the weight of the pituitary. If there was a difference in hormonal activity, it was less than the differences in the response of various individual fish.

In practice then, variations in size of pituitaries may account for differences in effectiveness of the injections if pituitaries are injected without weighing. It is suggested that pituitaries used for injections be combined, finely ground, thoroughly mixed, and weighed for injection to provide more uniform results.

Relation of pituitary weight to fish length and weight

We have demonstrated that the size of the pituitary in mature fish is a general indicator of its potential gonadotropic activity. We also endeavored to determine how the size of the pituitary varied with the size of fish. Pituitaries were removed

and weighed immediately after the fish were killed. Average pituitary weight for each length group of gravid goldfish was plotted against that group to provide the line of figure 1. It clearly demonstrates a straight line relationship. The regression equation for the grouped data is $Y=1.30-6.31$ and the coefficient of correlation is 0.892. However, there is considerable variation in pituitary size as related to length of fish, as indicated in the range of pituitary weights for each group of fish lengths (table 8). A small portion of this variation may be due to the fact that different amounts of the stalk and membranes were removed and weighed with the pituitary and that slightly different amounts of moisture remained on each pituitary after blotting.

A similar straight-line relation between fish length and pituitary weight is shown for the male and female channel catfish (fig. 2, based on data in table 9). The regression formula for males is $Y=1.55x-15.55$ and for females it is $Y=3.40x-44.44$. The coefficient of correlation for males is 0.887, and for females it is 0.817. When pituitary weight was plotted against fish weight (fig. 3) the regression equation was $Y=3.56x+4.12$ for males and $Y=8.14x-3.31$ for females. The correlation for males is 0.911 and for females it is 0.952. Thus, there is a slightly higher correlation between pituitary weight and fish weight than there is between pituitary weight and fish length.

TABLE 8.—*Relation of pituitary weight (wet) to fish length of gravid goldfish*

Total length of fish	Range of weights (milligrams)	Average weight (milligrams)	Number of pituitaries
5.0 inches	0.8	0.8	1
5.5 inches	1.0- 1.2	1.1	2
6.0 inches	0.7- 3.6	1.4	8
6.5 inches	1.5- 3.2	2.1	4
7.0 inches	0.8- 4.2	2.1	10
7.5 inches	2.0- 3.8	3.1	3
8.0 inches	0.9- 6.0	3.8	7
8.5 inches	1.2-10.2	6.0	5
9.0 inches			
9.5 inches	6.1	6.1	1

TABLE 9.—*Relation of pituitary weight to fish length of channel catfish*

[Only one fish was used in each length listed]

Length of fish	Weight of fish (pounds)	Wet weight of pituitary (milligrams)
Male channel catfish:		
15.4 inches	1.6	11.5
15.5 inches	1.5	11.0
16.2 inches	1.4	8.5
16.7 inches	1.7	12.0
17.8 inches	1.9	12.0
18.1 inches	2.4	8.5
18.1 inches	2.4	9.0
18.5 inches	2.5	15.0
20.8 inches	2.8	14.0
20.8 inches	4.0	15.0
22.4 inches	3.4	18.0
24.0 inches	5.6	25.0
25.0 inches	5.4	25.0
Gravid female channel catfish:		
13.8 inches	.9	4.5
14.5 inches	1.3	6.1
15.2 inches	1.2	6.3
16.0 inches	1.4	8.2
16.9 inches	1.6	13.3
17.6 inches	2.2	11.5
17.6 inches	2.2	12.4
19.3 inches	3.4	26.0

The ratio of the weights of fresh to the acetone-dried pituitaries for 26 female goldfish was 3.13:1 (24 percent of the wet weight). The ratio of the average wet weight of the pituitaries to the average weight of the gravid female goldfish was 1 milligram of pituitary to 34.9 grams of fish, or 13 milligrams per pound of fish. This ratio ranged from 5.7:1 to 26.9:1. The ratio of the wet weight of the pituitary in milligrams to the weight of female channel catfish in pounds was 6.2:1; for males 5.4:1 (table 9).

Even though the relative size of the pituitary varies phylogenetically, the fact does not appear to be of any practical significance to fish-cultural practices. We have used standard dosages based on milligrams of pituitary per pound of body weight of the recipient, regardless of the size, sex, and species of the donor. This practice may have to be changed for the pituitaries of certain species, e.g., river carpsucker and drum, as more information is gained, since our data indicate that the pituitaries of these species are low in activity.

Pickford and Atz (1957) reviewed the literature concerning the percentage of

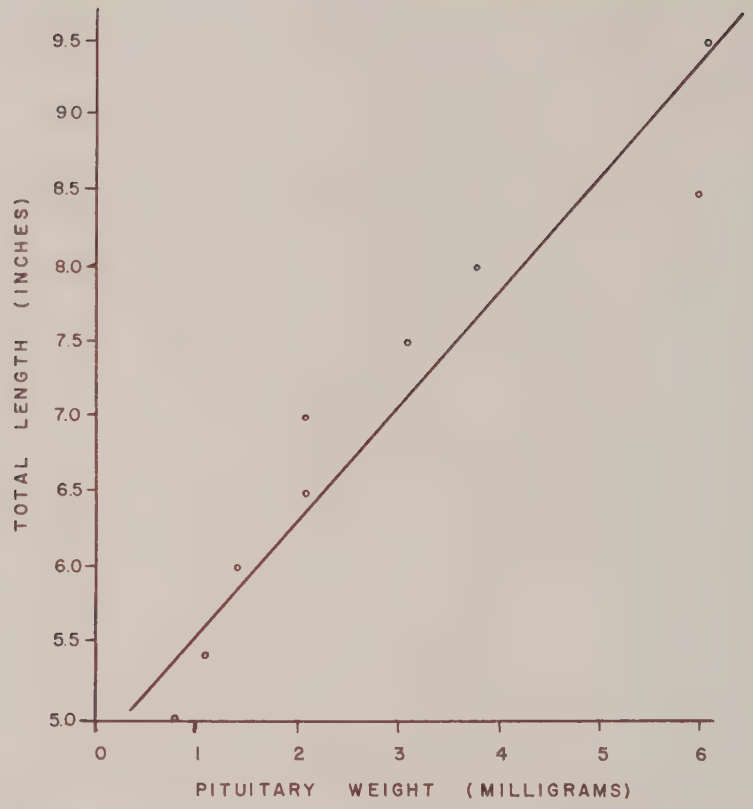

FIGURE 1.—Relation between pituitary weight and total length of gravid female goldfish.

weight loss in drying pituitaries in acetone and found that the defatted, dry weight represents 13 to 17 percent of the wet weight of the pituitaries of four different teleosts, which, it might be noted, belong to three different orders, two of the species being freshwater, one anadromous, and one marine.

Latent period

The time of response from injection to ovulation (the latent period), provided that it is rather constant, has practical significance in fish culture. Knowledge of this latent period permits more efficient hatchery operation insofar as the use of fish, men, and equipment are concerned. We have noticed that goldfish carefully selected for their apparent advanced spawning condition and injected in the afternoon invariably spawn early the next morning if the water temperature is about 70° F. At lower temperatures, a somewhat longer period is required.

These observations raised the question of whether the fish respond in a given interval of time or whether the exogeneous gonadotropins ready the fish physiologically and they spawn early the next morning when light and other environmental conditions are favorable for normal spawning. The following experiment was designed to answer this question.

Four female goldfish were each injected with 2.6 milligrams of acetone-dried carp pituitary collected in December. At 2-hour intervals, other groups, each of four fish, were similarly injected. This proce-

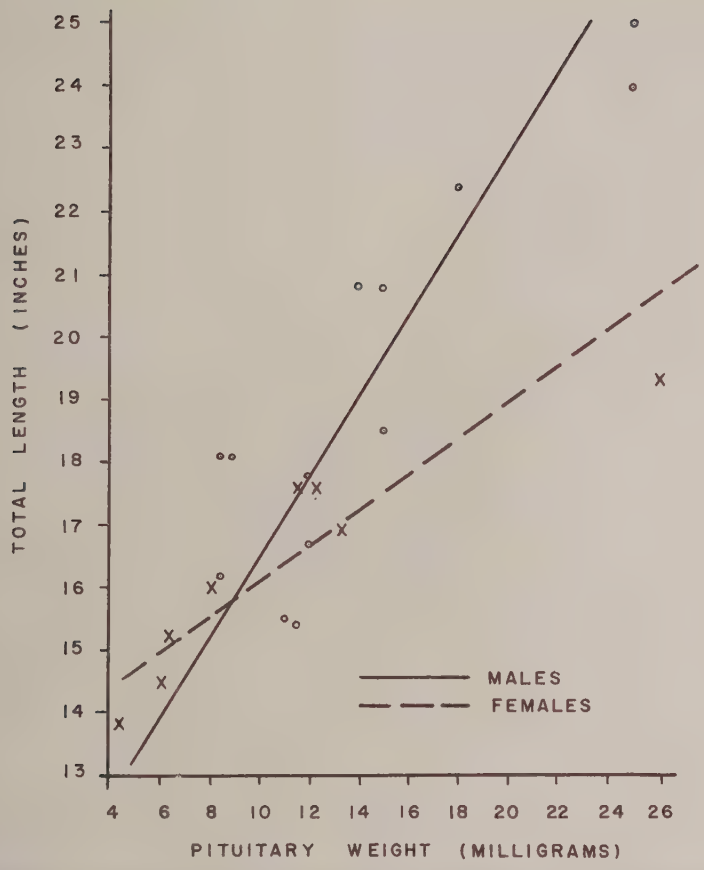

FIGURE 2.—Relation between pituitary weight and total length of male and female channel catfish.

dure was continued for a 12-hour period. All injected fish were examined every 2 hours. Presumably, if goldfish responded at a given time after injection, the fish which we first injected would spawn after that interval of time, and the others would spawn each 2 hours during the next 12 hours. If they spawned at a particular time of day, there would be no 2-hour pattern; the fish would spawn only in the morning.

No eggs could be hand-stripped from the selected fish prior to the pituitary injection. On subsequent attempts, if a few eggs could be stripped, the condition was designated as "early ovulation" (table 10). In other instances, hand-stripping released a considerable number of eggs which were closer to the color and texture of ripe eggs; this condition was designated as "incomplete ovulation". Only when the eggs could be stripped from the fish with slight pressure was the fish considered to be fully ripe and its condition rated as "complete ovulation". Admittedly the classification is a matter of judgment, but the four people who worked in the laboratory readily agreed upon the condition of the fish and eggs in all cases.

Of the 28 fish injected throughout the course of the experiment, all fish that ovulated did so within 12 to 18 hours after injection. One fish responded in 12 hours, seven in 14 hours, nine in 16 hours, and

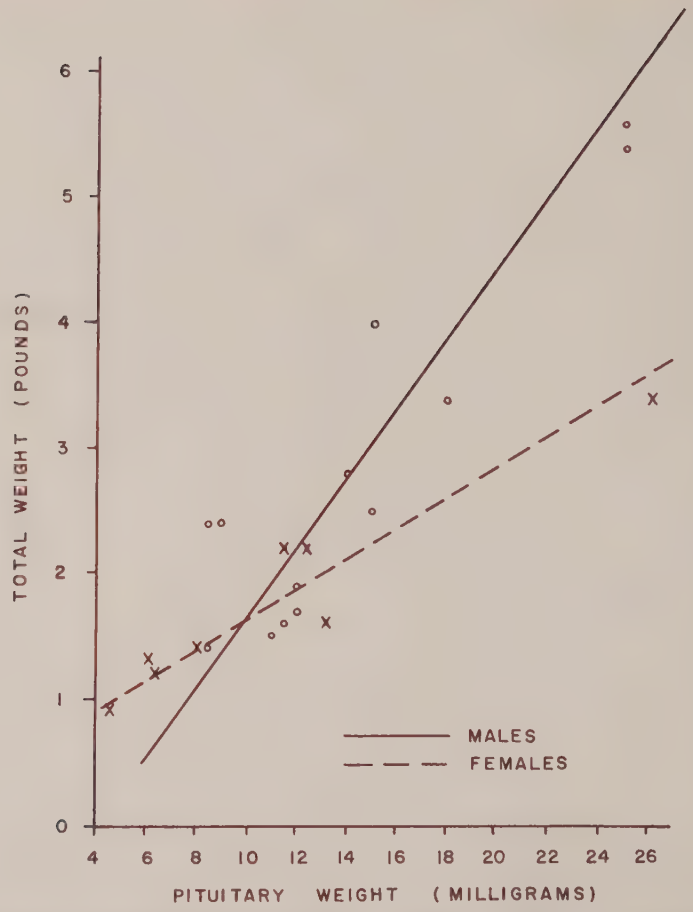

FIGURE 3.—Relation between pituitary weight and weight of male and female channel catfish.

two in 18 hours. Sixteen fish, about 90 percent, responded between 14 and 16 hours at a temperature of about 72° F. Nine fish did not ovulate.

The fact that 90 percent of the females responded to hormone injections after 14 to 16 hours is significant to the fish-culturist. For example, if goldfish are injected during the late afternoon, spawning should occur during the early morning hours when the sun is coming up and when the goldfish normally spawn. However, if one desires spawn during other hours, fish can be injected at times calculated to give spawn at any hour which might be advantageous to the culturist.

It is not particularly significant that 9 of 28 females did not ovulate, since the fish employed were not selected to be completely ripe. The pituitary method of spawning as used in this experiment was not intended to ripen fish, but merely to precipitate ovulation in ripe fish. The spawning of unripe fish is another problem and involves different techniques.

The length of the latent period of goldfish varies with temperature. If the fish were ripe enough to respond at all, the latent period increased from 12 to 25 hours as the temperature decreased from 85° to 52° F., the range of temperatures at which goldfish were spawned in our ex-

TABLE 10.—*Latent period for female goldfish injected with 2.6 milligrams of carp pituitaries at 72° F.*

Size	Time of injection	Number of hours from injection of pituitaries to spawning		
		Early ovulation	Incomplete ovulation	Complete ovulation
7.25 inches	0815		14	16
8.5 inches	0815			16
8.0 inches	0815	12	14	
7.25 inches	0815	12	14	18
8.0 inches	1015			16
7.75 inches	1015	10		18
7.5 inches	1015	10	24	
7.25 inches	1015	14		16
8.75 inches	1215			16
8.5 inches	1215			14
6.75 inches	1215	12		14
7.5 inches	1215			16
7.25 inches	1415			16
7.0 inches	1415		20	
7.25 inches	1415	4	8	
6.75 inches	1415	10	18	
8.25 inches	1615	2		14
7.75 inches	1615	2	18	
9.0 inches	1615			14
7.5 inches	1615		18	
7.5 inches	1815			16
7.0 inches	1815	8		16
8.0 inches	1815			12
6.25 inches	1815	2	16	
8.0 inches	2015			
8.5 inches	2015			14
7.5 inches	2015			14
6.5 inches	2015			14

periments. If certain goldfish require considerable ripening and more than a single standard injection is necessary to precipitate spawning, the time of response from the first injection is probably more contingent upon the amount of maturation required than on the dosage level, if the level is equal to or above the standard dose determined for ripe females in the same lot of fish. This statement is made in light of the fact that the strength of the injection does not seem important to the induction of spawning or to the latent period of ripe goldfish once a certain level or threshold is reached. Since gonadotropins are probably excreted or inactivated, the excess quantities therefore would not stimulate ripening in direct proportion to the dosage level, but would be somewhat less than proportional. The fact that the length of the latent period was fairly constant probably means that the threshold dosage existed, and the injected fish as a group were of similar physiological condition. One thing that should be noted here is that different groups of goldfish do have different quantitative requirements for a threshold dosage (table 3), which again emphasizes the importance of the spawning condition of the recipient and the experience of the fish-culturist in judging that condition.

Another point to be made in regard to the variable latent period (12 to 18 hours) is that after 18 hours no fish continued development to the "complete" ovulation stage, suggesting that the absorption of hormones from the 2.6 milligrams of pituitary was virtually complete after 18 hours. In another paper (Sneed and Clemens, 1958), our data on channel catfish suggest that the level of gonadotropins necessary to produce spawning was maintained more effectively by small dosages (every 8 hours) than by larger dosages less frequently given (24 hours or longer).

Effect of exogenous gonadotropins on fertility and development

Pickford and Atz (1957) stated in connection with their review of Russian literature that "there is ample evidence that improper application of the method of inducing ovulation by pituitary injection can yield inferior sex products." "Improper application" means too large a dose, and "inferior sex products" means infertile eggs or sperm, reduced viability, the incidence of monsters, and in the case of sturgeons, parthenogenetic development of the eggs. However, the Russian data are not clear cut, and Russians themselves argue the point.

No such effects have been detected in incubating and hatching the eggs, or in raising the fingerlings from over 200 channel catfish spawns obtained by pituitary injections. Some decrease in fertility (1 to 5 percent) was observed when the same male was used to spawn two or more females, and in the spawns from fish early or late in the season. Such instances of infertility can be accounted

TABLE 11.—*Fertility of eggs from carp and goldfish injected with acetone-dried pituitaries 15 to 24 hours before handstripping.*

Female	Male	Percent fertility	Remarks
Goldfish	Goldfish	99	Development followed to hatching.
Goldfish	Goldfish	91	Development followed to hatching.
Goldfish	Goldfish	92	Development followed to hatching; unfertilized eggs stored in plastic bag 1 hour before fertilization.
Goldfish	Carp	75	
Carp	Goldfish	97	Eggs stripped into Ringer's solution immediately before fertilization.
Carp	Goldfish	91	
Carp	Carp	97	
Carp	Carp	90	Sperm stored in Ringer's solution before use.
Goldfish	Carp	80	Unfertilized eggs stored in plastic bag in refrigerator 1 hour; sperm stored 4 hours in Ringer's solution.
Goldfish	Carp	80–85	Unfertilized eggs stored in refrigerator 1 hour.

for on the basis of incomplete development early in the season and natural deterioration of eggs late in the season. It was not uncommon for the eggs to be inferior in the first few ovipositions, and on occasion these were eaten by the spawning fish. The lowered fertility was never great enough to be of practical significance; and it was never attributed to hormone injections, except when fish were hormone-spawned before their normal spawning season, or late in the season.

Excellent fertility was demonstrated by handstripping carp and goldfish of both sexes, which were previously injected with acetone-dried pituitaries (table 11). Similar results were obtained when the injected fish were allowed to spawn on mats.

Exogenous gonadotropins can be used successfully to spawn fully developed females. Inferior results, as reported in the literature, probably involve extremely large doses of pituitaries, faulty techniques, or the use of unripe or delayed fish. From a fish-cultural standpoint, the understanding of why some fish are refractive to hormone injections is more important at the present than is an explanation of slightly harmful effects of gonadotropins on fertility and development.

USE OF PITUITARY MATERIALS

A review of the attempts to spawn fish by hormones reveals a number of techniques which produce varying degrees of success and failure. The reason that a proper and proved technique may produce negative results lies to some extent with the qualifications of the investigator who usually is either an endocrinologist who knows almost nothing about fish culture or a fish-culturist who is rather uninformed about endocrinology. For this reason, we regard only positive results in the reports of induced spawning experiments as truly significant. Sometimes even the positive results need to be regarded with caution since fish occasionally can be chemically or physically shocked into spawning.

The condition of the female fish at the time that induced spawning experiments are conducted is of utmost importance. The method presently used and discussed in this paper concerns spawning of ripe fish only. Within relatively narrow limits, fish can be hastened into spawning by pituitary injections. Likewise, fish slightly delayed in spawning, or refractive to spawning in their natural habitat, can be spawned by these methods. The manipulation of fish beyond these narrow limits is another problem.

The best method of selecting fish for advanced sexual development is to examine the larger fish in the hand; smaller (minnow-sized) fish can be examined in a quart jar. The assessment of the females' physiological receptivity to gonadotropins was based on a combination of the following characters: (1) Abdominal distention by the ovaries, preferably after the digestive tract has been emptied; (2) con-

dition of the genital opening; (3) color; (4) degree of abdominal flaccidity; (5) behavior.

The assessment of the degree of sexual development of the male is not usually a major problem. During the spawning season, males of many species often show sexual dimorphism, and any male observed in breeding color was assumed to be ripe. If sexual dimorphism does not exist, and it is difficult to determine ripe males from unripe females and immature individuals, the fish of undetermined sex can be stocked at a ratio of two or more per gravid female in an effort to assure the presence of some mature males in the tank. These fish should be similar in size to the selected gravid females. In those species that pair, the behavior of the male frequently indicates whether or not he is a potential spawner. His positive or negative attitude toward the female or to other males often reflects his sexual development. A thorough knowledge of the natural spawning behavior and conditions for the particular species makes it much easier to select fish that can be induced to spawn. Both males and females are usually injected for handstripping procedures, but otherwise only the females receive gonadotropins. For species that require the more specific environments for spawning, such as the nest builders, it may be necessary to provide certain facilities such as vegetation, gravel, rocks, cans, or flowing water, since stimuli from the environment may also play an important role in the proper metabolism and activity of the injected hormones.

Other important factors of general technique to be considered include dosage and handling. It usually saves time and labor to give doses above the minimum required for spawning, rather than to administer a subminimal dose. The evidence indicates that once the spawning dose is reached greater amounts of pituitary do not change the results (table 3).

Our general technique varied with the size of the fish. Minnow-size fish (2 to 3 inches long) were usually anesthetized in 1:10,000 MS 222 and were held in the folds of a net. They were injected intraperitoneally with 0.1 to 0.2 milligrams of pituitaries in 0.1 to 0.2 milliliters of distilled water which contained 100 to 200 units of penicillin. If greater volumes were injected, the fluid was more likely to be lost from the body cavity after the needle was withdrawn. Also, greater volumes produced an edema and peritoneal inflammation, perhaps because the internal pressure was too great for the welfare of the fish, or the volume of fluid was too great for complete absorption. For these small fish, a needle size of 26 or smaller was used. For larger fish, needle sizes from 19 to 21 were satisfactory and did not often clog. Luer-lock hypodermic syringes, which have a locked needle, will prevent the loss of materials if the needle clogs. Large fish were not anesthetized. They were injected intraperitoneally with pituitary dosage of 2 milligrams or more per pound of body weight, with 1 milliliter or more of distilled water, and 10,000 units of penicillin if multiple injections were necessary.

Suitable facilities and proper spawning places (such as well-aerated running water, aquaria, troughs, pens, or ponds) must be provided, the type depending on the species to be spawned. If a question of water temperature arises, a temperature approximating that at which spawning occurs in nature is used. If there is danger of the eggs being eaten, some species (e.g., zebra fish) can be spawned over netting through which the eggs can settle and be separated from the parent fish. Other species (goldfish, carp, etc.) can be spawned on mats which are periodically moved to hatching ponds or troughs. The egg masses of the catfishes are easily moved to mechanical hatching facilities immediately after spawning is complete.

An account of our experience in spawning twelve species of fish is given in the following sections and is summarized in table 12. It may appear to the reader that in the following sections that not enough attention has been given control experiments. In those species (goldfish, carp, channel catfish and golden shiner) that were spawned on a large scale, control groups were usually not set up at this stage of our investigations, since many previous controlled experiments had indicated that these species did not often spawn in confined spaces (aquaria, holding troughs, etc.) without pituitary injections.

In the case of the zebra fish, too few controls were used to demonstrate a clear-cut difference between the response of injected and uninjected fish. Since this fish readily spawns in small containers, the effect of gonadotropins never will be as pronounced.

The information on the spawning of the remaining species should be considered as preliminary, since only a few fish have been spawned. When only a few fish are involved, negative controls have little meaning, since the fish are so individualistic in their physiological condition. Only random injections and random controls from a particular population, and then only if many individuals are used, give statistical significance to controlled experiments involving induced spawning in many species of fish. For such species as the channel catfish, which, when held captive in small aquariums, does not spawn unless it is injected, controls appeared superfluous after this fact was definitely established. Also, for all species with which we worked, control information was accumulated inasmuch as similar, uninjected fish were almost always maintained in the laboratory under practically identical conditions, although formal controls were not designated.

The response of a particular species of fish to pituitary injections varies with the gonadal development of the fish. Very gravid females respond much more readily than fish less well developed. In other words, it is easy to induce spawning during the regular spawning season, the fish often spawning with one injection; whereas fish injected several weeks before their regular spawning season may require several injections over a period of hours or days in order to precipitate the spawning act. Usually several injections must be used to spawn fish which have been delayed past their normal spawning period, but spawning cannot be obtained if degeneration of eggs has begun. Usually delayed fish are refractory, but quite often normal eggs can be procured if several low-level dosages are give. For example, we have spawned carp as early as February and as late as July 14 by use of the pituitary injection method, and Mr. Mayo Martin (personal communication) spawned carp in September in Arkansas. There are records in the literature of cyclic-spawning fish having been spawned during practically every season of the year (Hasler and Meyer, 1942; Kawamura and Otsuka, 1950; Hasler, Meyer, and Field, 1939; Ball and Bacon, 1954). Apparently, the major requirements for successful induced spawning are that fish contain well-developed eggs and that the injected pituitaries contain the components necessary to precipitate the spawning act or to produce ovulation. In many species (carp, goldfish, channel catfish, green sunfish), sperm is available in at least some males most months of the year (see also Pickford and Atz, 1957, p. 216).

Chronic injections of pituitary materials at dosage levels normally used to induce spawning in a particular species can be harmful to fish that contain small, immature eggs. For example, 1-year-old female largemouth bass (*Micropterus salmoides*) whose ovaries contained mostly immature, the amount of pituitary which tracted chum salmon pituitaries in choles-

TABLE 12.—*Summary of induced spawning experiments for twelve species of fish*

Species	Size of recipient	Number spawned	Month	Water temperature (°F.)	Donor species	Dosage	Number of milliliters	Number of injections	Facilities	Can be hand-stripped?
Goldfish (*Carassius auratus*).	3–12 inches	Thousands	Mar.–June	65	13 species	1 gland	1.0	1–2	Aquariums, troughs, ponds, mats.	Yes.
Carp (*Cyprinus carpio*)	1 lb	Hundreds	Mar.–June	75 (65–85)	Carp	1 gland (1–5 mg).	1.0	1–2	Troughs, ponds, mats	Yes.
Golden shiner (*Notemigonus crysoleucas*.	3–5 inches	Hundreds	May	68	Carp	1/20–1 gland	0.2	1	Aquariums, ponds, mats	Yes.
Emerald shiner (*Notropis atherinoides*).	2½–3½ inches	1 to 3	July	76	Buffalo	1/10 gland	0.2	1	Aerated aquariums; fish over netting.	Not known.
Mimic shiner (*Notropis volucellus*).	2 inches	1 to 8	June	76	Carp	1/10 gland	0.2	1	Aerated aquariums; fish over netting.	Not known.
Redfin shiner (*Notropis umbratilis*)	1½–2 inches	1 to 4	July	76	Buffalo	1/10 gland	0.2	1	Aerated aquariums; fish over netting.	Not known.
Channel catfish (*Ictalurus punctatus*).	0.7–8.2 pounds	200	May–June	70 (70–85)	5 species (see text).	2 mg./lb	1.0	3	Aquariums, troughs, pens	No.
Flathead catfish (*Pylodictis olivaris*).	5.0 lb	4	July	75	3 species (see text).	2–6 mg. per lb	1.0	3–4	Aquariums, troughs	No.
White crappie (*Pomoxis annularis*).	10–12 inches	4	May	61	Carp, Saline extract.	4 mg	1.0	1–2	Troughs, mats	Yes.
Rock bass (*Ambloplites rupestris*).	5–7 inches	2	July	76	Buffalo	2 mg	1.0	2	Aquariums, gravel, running water.	Not known.
Zebra fish (*Brachydanio rerio*).	1–2 inches	Several	Dec.–May	80	3 species (see text).	0.6 mg	0.2	1	Aerated aquariums; fish over netting.	Yes.
White bass (*Roccus chrysops*).	9–12 inches	2	June	72	Carp	0.5 and 2 mg	1.0	2	Trough, mats	Yes.

terol pellets (prepared by Dr. Arthur Rinfret and supplied to us by R. E. Burrows). These fish became nervous after 5 or 6 days, began to lose mucus from the body, developed whitish spots, and jerked violently when handled. The muscles of the fish felt hard, as though the fish had been dehydrated. On the second day the fish gave off large quantities of urine when stripping tests for eggs were made, and would often flex into a rigid sigmoid curve when handled. Fish responded similarly to large amounts of fresh pituitary given frequently over a 24-hour period, or when large doses were administered daily for 6 or 7 days. We have noticed a few identical responses in other species including crappie, goldfish, carp, and zebra fish. There are several possible explanations for such phenomena:

1. When gonads are poorly developed or immature, the amount of pituitary which we injected disrupted metabolic processes of the fish, i.e., the injections constituted a pharmacological overdose.

2. The extract which we used and the raw pituitaries contained products which were toxic to the fish.

3. The fact that the fish appeared dehydrated and that the urine was increased suggests that hormones had a diuretic effect on the fish.

The toxicity of some pituitary materials to immature fish may possibly be due to ACTH, as suggested by Roger E. Burrows (correspondence).

Two results of our research suggest that the hormonal requirements of the two groups of fish are different quantitatively and/or qualitatively: (1) Sexually mature fish respond in an apparently normal fashion to dried, whole pituitary and to pituitary extracts; (2) immature fish respond abnormally to similar materials.

Goldfish *Carassius auratus*

Inasmuch as thousands of goldfish have been induced to spawn in various conditions at a number of hatcheries over a period of years the following method may be considered as standard. Almost identical techniques have been used with many carp and golden shiners.

During the regular goldfish spawning season from March to June, well-developed females 3 to 12 inches in total length usually spawn or can be hand-stripped within 12 to 20 hours after a single injection of pituitary taken from a fish of equal weight, or an injection of dried pituitaries of 1 to 5 milligrams per fish (2 to 3 milligrams per pound of body weight), depending on size. In practically all cases, fresh or acetone-dried materials in 1 cc. of distilled water were injected intraperitoneally at the axil of the pelvic fin of the female. Males were injected only to increase the flow of milt when hand-stripping was practiced.

Goldfish have responded to pituitaries from 14 species (table 4). Fish were spawned at temperatures from 52° to 85° F. For best results, water temperatures should be between 60° and 80° F. and an adequate supply of oxygen must be present. Goldfish were usually injected with the appropriate amount of pituitary materials in the afternoon, the dosage having been determined a day or two previously with the injection of some 10 or 15 females with varying amounts of the pituitary materials to be used. After the females were injected they were placed in concrete or metal troughs or in small earthen ponds with an equal number of males which may or may not be injected. Spanish-moss spawning mats were placed around the edges of the troughs and ponds, as described by Prather, Fielding, Johnson, and Swingle (1953). Such fish usually began spawning about daylight on the following morning and completed spawning by 10 or 11 a.m. Sudden drops in temperature from 70° to 50° F. usually did not completely inhibit spawning, but only delayed it. When spawning was

finished, the mats were moved to a hatching pond which had been filled recently and was therefore free from predators. If the water had been standing for several days in the pond, or if runoff or pond water was used, this water was treated with 2.5 p.p.m. Lexone 2 or 3 days before stocking. This treatment effectively removes crayfish, backswimmers, some parasites, copepods, and predatory insect larvae. If properly used, Lexone will not be detrimental to the fertility of the eggs. The required number of eggs should be placed in the hatching pond in a single day, if possible, so that all fry will be the same age. Consequently the growth of fry will be more uniform, and some parasites, such as *Lernea*, will not be transferred from brood stock to fry. Any larvae of this parasite which might be transferred on the mats will likely die before the eggs hatch, since these larvae will live only about 24 hours in 2.5 p.p.m. Lexone and only a few days unattached to a proper host.[1]

An alternative method for the culture of this species involves the hand-stripping of eggs and sperm after injection. Instead of placing the female in spawning troughs or ponds, they are kept in holding tanks separated from males. Males are injected with pituitary materials to increase the amount of seminal plasma and provide more milt for the worker to use. The morning following the injections, the females are examined to determine their degree of ripeness. Those females that are ready to strip are separated into buckets or other convenient holding facilities. When several fish are ready to strip, the eggs are removed in a manner similar to that used for trout and pike. Each female is wiped dry with cheesecloth or toilet tissue, and the eggs are stripped into a plastic container, to which they do not stick as they do to glass or enamel pans. Great care should be taken to keep the eggs and milt perfectly free of water until completely mixed. The milt and eggs should be well mixed with a nylon bristle brush before any water is added. The procedure to this point should not be hurried, since the sperm of goldfish do not normally become motile until water is added.

We have kept goldfish eggs in a dry state outside the body of the female 1 hour (table 11) and found that they are totally receptive to fertilization. Also, goldfish eggs have been stored 1 hour in a sealed plastic bag in a refrigerator without serious loss of fertility (table 11). Shortly after mixing the eggs and sperm, small amounts of water are dripped from the finger into the eggs and sperm. After sufficient water is added, the eggs will begin to clump and adhere to each other. Slightly before much clumping begins, the eggs should be lifted with a nylon bristle paint brush and spread on spanish moss mats placed 4 to 6 inches under water in a holding trough or other convenient utensil. As the eggs are shaken from the brush, the water about the mat should be vigorously agitated to disperse the eggs over the mat. Also, the eggs can be poured from the plastic container and dispersed over the mat by vigorous agitation of the water. With a little practice the operator can achieve a dispersion of eggs similar to that attained by the fish under normal spawning conditions. Good dispersion of the eggs is essential; any dead eggs will become covered with fungus and smother adjoining living eggs if they are left in clumps or masses.

After the eggs have been placed on the mats, either of two incubation methods may be used. The mats of eggs may be placed in hatchery rearing ponds as de-

[1] John J. Giudice, Control of *Lernea carassii* Tidd, a parasite copepod infecting goldfish in hatchery ponds, with related observations on crayfish and the fish louse *Argulus* sp. Master's thesis, University of Missouri, 1950.

W. M. Tidd, Studies on the life history of a parasitic copepod, *Lernea carassii*. Doctoral thesis, Ohio State University, 1947.

scribed above, or they may be stored in regular hatchery receiving troughs which have flowing water. If many dead eggs are present, they may be flush-treated with malachite green at about 2 p.p.m. Water inflow should be adjusted so that the chemical is flushed out within an hour. When the eggs are eyed they may be moved from the troughs to a growing pond which has been properly prepared for fry. Moving the eggs at the eye stage is much easier than after hatching, since the fry of goldfish are small and delicate. We have never tried to feed or raise carp or goldfish in troughs. Trough-rearing may be feasible in this country in small hatcheries which rear fancy goldfish. The above methods have been used for several years to raise goldfish at commercial fish hatcheries with which we have been associated as employee or consultant. Also, these techniques have been used to raise young goldfish for food for bass at the National Fish Hatchery, Tishomingo, Okla., for the past four seasons. In one instance 675,000 goldfish weighing 2,925 pounds were reared in a 0.7-acre pond in 5 months. This concentrated population of fish did not suffer any serious diseases or mortality (Cozort, 1955).

Carp (*Cyprinus carpio*)

The technique for spawning carp is almost identical to that of goldfish (table 12) except that, in our experience, carp are refractory to heteroplastic injections. We have injected as many as 20 goldfish pituitaries in carp with no results. Similar doses of buffalo and catfish pituitaries also have been tried with negative results, whereas a single carp pituitary will induce female carp of the same group of fish to spawn. Ordinarily, carp are difficult to spawn in small earthen ponds or hatchery troughs; but under the influence of exogenous pituitary hormones, they can be effectively spawned in such ponds or troughs, or they can be hand-stripped as described above for goldfish.

Golden shiner (*Notemigonus crysoleucas*)

The technique described for small fish was used for this species and the three notropids following. The fish were anesthetized, held in the folds of a net, and the injection was made through the mesh. Female golden shiners received amounts of pituitary varying from one-twentieth to a whole pituitary from a 1-pound carp. One-tenth of a pituitary for gravid females 3 to 5 inches long is recommended. The eggs are adhesive and Spanish-moss mats were provided for spawning. If hand-stripping was employed the procedure outlined above for handling the adhesive eggs of goldfish was followed. At a water temperature of 68° F., spawning was induced in about 15 hours after a single injection. In addition to the above experiments, we have worked with commercial bait raisers, and the pituitary method was applied to large-scale operations. The brood fish were usually large, and anesthetics were not necessary. Most of this work was not subject to controls, but the method definitely appears suitable for the production of large numbers of golden shiners with fewer brood fish and less possibility of disease. Excellent production and uniformity in size of the bait minnows were achieved at Sulphur Fish Hatchery, Sulphur, Okla., when 200 brood females were injected and allowed to spawn in a natural situation in an earthen culture pond. Similar ponds stocked according to previously recommended methods produced inferior results.

Emerald shiner (*Notropis atherinoides*)

The emerald shiner was induced to spawn when injected with the water extract of one-tenth of an acetone-dried gland (estimated from 1 to 2 milligrams) from a buffalo (table 12). These shiners were collected from Lake Erie during the

second week of July. Each of nine fish, 2 to 3 inches in length was anesthetized in 1:10,000 MS 222 for 1 minute and then was injected intraperitoneally with 0.1 milliliter of distilled water containing 100 units of crystalline penicillin-G plus the pituitary. The distended abdomens of three of the nine fish indicated that they were gravid females. One fish died the following morning; the others were alive and appeared normal. They were placed together in water at 76° F. in a 15-gallon aquarium. A piece of ⅛-inch mesh nylon net was hung between the fish and the bottom of the aquarium to prevent consumption of the eggs by the parent fish. The shiners spawned 20 to 24 hours after injection, and fertile eggs were found loose and scattered on the slate-bottomed aquarium. They were observed until developed to the primitive streak stage. No attempt was made to put the eggs in a proper hatching environment. Fungus attacked the eggs, and none hatched.

Mimic shiner (*Notropis volucellus*)

The dosages (table 12), injection technique, and aquarium setup was the same as for the emerald shiner, with the exception that the pituitary was from carp. Fish were collected from Lake Erie in the third week of June, and 18 fish about 2 inches long were injected. Six of these were known to be gravid females, but could not be hand-stripped before injection. No fish were lost within a 24-hour period after injection, but two died within the next 3 days. The eggs were loose and scattered, both individually and in clumps, over the bottom of the aquarium. They were observed to be fertile, but their development was not followed.

Redfin shiner (*Notropis umbratilis*)

Six male redfin shiners in breeding color were chosen and were not injected. Four gravid females were injected at the same dosage as the emerald shiner (table 12) about 9 p.m., July 17, and the males and females were placed together in a 5-gallon aquarium. The following morning between 7 and 10 o'clock spawning began and continued until about 4 p.m. It appeared that one male dominated the aquarium, driving the others to the surface to hide in the folds of the net while he spawned with the females. The dominant male was much more brilliant in color than the others, although they were all about the same color at the beginning of the experiment. (Later, in another experiment, a breeding male was placed with an injected female and within 2 hours the color on his fins had become much more intense.)

Eggs were deposited in a small pile in one end of the aquarium, and later in another pile at the other end of the aquarium. The selection of a new position at the far end of the aquarium away from the observer may have been caused by disturbances. Since the male was separated from the eggs by netting, it could not be determined whether or not he attempted to tend them. He moved throughout the aquarium. The eggs were left in the bottom of the aquarium. Although many of them became infected with fungus, several uninfected eggs hatched during the third day.

Channel catfish (*Ictalurus punctatus*)

The induction of spawning of the channel catfish by the injection of pituitaries has been previously described (Sneed and Clemens, 1960), and an abstract of this report is presented here for the sake of completeness.

Seventy-four pairs of channel catfish were injected with fish pituitary and induced to spawn in aquariums. Only the females were injected. Ten-gallon aquariums were the most satisfactory containers for spawning fish up to 2½ pounds in weight. Larger fish required proportionally larger containers. Peritoneal lesions

and inflammation which often accompany intraperitoneal injections appeared to be controlled by including 10,000 units of crystalline penicillin-G with each injection.

Pituitaries from carp, buffalo, flathead and channel catfish, and gar were successfully used. There was little difference noted in the potency of the pituitaries from these different species, regardless of the month of collection. The total amount of acetone-dried pituitary material required to spawn channel catfish varied from 3 to 32 milligrams per pound of body weight of the recipient. Most females required 3 injections at 2 milligrams per pound of body weight. The total number of injections required varied from 1 to 28, with the average being 3. The period of time from the last injection to spawning varied from 2½ to 72 hours, but most fish began spawning 16 to 24 hours after the last injection.

Females believed to be refractory to spawning in the hatchery ponds and in their natural habitat were induced to spawn in aquariums by pituitary injections.

Flathead catfish (*Pylodictis olivaris*)

We have spawned four pairs of the flathead catfish, and the general technique was the same as for the channel catfish. The females were placed with males of similar size in 55-gallon aquariums, 24 x 25 x 14 inches. One pair was spawned in a cement trough. The fish were obtained from Lake Texoma on July 5 and injections began 2 days later. The data in table 13 outline the treatment used on individual fish to induce spawning and should serve as a starting point for those who wish to work with this species. Before flathead catfish can be spawned with any degree of success, some experience in selecting females is needed. Ripe females are usually darker in color than males, but were difficult to discern by external examination in the fish which we handled. The best criterion for evaluating the spawning condition of the females is the appearance of the genitalia. The genital papillae should be slightly raised, somewhat reddish, and the genital opening slightly dilated.

When examined in June, the ovaries of flathead catfish brood stock kept all winter in a hatchery pond were so poorly developed that we felt the fish could not be spawned. However, a hatchery in southern Texas that kept part of our brood stock through the winter was able to spawn one pair. Similar reports (personal communication) of difficulty in rearing flathead catfish brood stock have come to us from Arkansas and Alabama. Water temperatures and failure of the fish to feed properly in hatchery conditions on artificial foods may be major deterring factors in the sexual development of this species.

A local minnow breeder successfully spawned five pairs of flatheads with injections of pituitary materials according to our recommendations (Nos. 5–9 in table 13). He used buffalo, carp, and flathead pituitaries at dosages of 2.5 to 4 milligrams per pound of fish every 24 hours. Two of the females spawned with one injection, which suggests that flatheads respond to gonadotropins in a manner similar to other species if they are physiologically ready to spawn.

White bass (*Roccus chrysops*)

Two female white bass secured from the Red River below Lake Texoma on June 12 contained unspawned eggs, although the white bass normally finishes spawning before this time. These fish were taken to the hatchery with males of similar size. Both males and females were injected with ½ milligram of acetone-dried carp pituitary and placed in hatchery holding troughs, with Spanish-moss mats placed on the bottom and around the edges of

TABLE 13.—*Injection records of nine successful spawns of flathead catfish*

Fish	Number of injections	Donor species	Dosage (milligram per pound in 24 hours)	Female weight (pounds)	Remarks
No. 1	4	Flathead	4.0	9.0	Ovulation of a few eggs occurred without spawning; different male added before spawning was achieved.
No. 2	4	Buffalo	2.0	15.5	Spawned in cement tank.
No. 3	3	Carp	6.0	6.0	Partial spawn.
No. 4	4	Carp	2.0	6.8	Partial spawn.
No. 5	3	Buffalo and carp		Est. 10.0	240 milligrams of B-12 with first injection; spawned in barrel in cement trough.
No. 6	5	Buffalo and flathead.	2.7	17.2	
No. 7	1	Buffalo	2.5	6.0	Collected in spawning condition, June 10, from Washita River; spawned in barrel in cement trough.
No. 8	1	Buffalo	2.5	1.8	Collected in spawning condition, June 10, from Washita River; spawned in barrel in cement trough.
No. 9	2	Buffalo	4.0	4.0	Collected in spawning condition, June 10, from Washita River; spawned in barrel in cement trough.

the trough (table 12). Seventeen hours later the fish were examined and were not ready to hand-strip, nor had any spawning occurred in the trough. The females were injected a second time with 2 milligrams of acetone-dried carp pituitaries and spawning occurred in the trough within 3 hours. Most of the spawn was placed on the mats, but there were many eggs on the sides and bottom of the troughs where the mats did not cover the entire surface. Had the bottom been completely covered with mats, 90 percent of the spawn could have been collected. Microscopic examination at the end of 12, 24, and 48 hours revealed about 60 percent of the eggs were fertile. These eggs were not observed further. One mat of eggs was placed in an earthen pond, but no fry were found, which may have been due to the fact that snails (*Physa*) soon ate the eggs.

White crappie (*Pomoxis annularis*)

Four pairs of white crappie were spawned in a cement trough containing Spanish-moss mats, and with a small flow of water running through the trough (table 12). One female received intraperitoneally one cholesterol pellet containing 1½ milligrams of an extract of chum salmon pituitary. At the end of 15 hours the genital pore was large and red; and at the end of 48 hours, a few eggs could be obtained by hand-stripping. This female was then injected with 4 milligrams of acetone-dried carp pituitary and spawned the following night when the water temperature was 61° F.

On another occasion, three pairs spawned within 30 hours after a single injection of acetone-dried carp pituitary. The eggs successfully hatched and the fry appeared normal.

Rock bass (*Ambloplites rupestris*)

A series of failures to induce spawning in nest building centrarchids has included the largemouth bass, bluegill, green sunfish, and rock bass. The information from these failures and from the successful spawning of two pairs of rock bass suggests a possible approach to a successful technique.

1. The condition of the male seems to be more important than in other species. It is the male that builds the nest. If the male does not initiate this behavior, then apparently spawning will not take place. For this reason, it may be good practice to inject the male, unless the male has been observed to exhibit some prespawning behavior.

2. The condition of the female is more difficult to assess because the genital openings reveal little concerning gonadal condition, and outward appearances of ovarian development can often be misleading.

3. Specialized environmental conditions

may have to be provided to give the necessary release to trigger the spawning. In the past, in spawning cyprinids, ictalurids, and serranids, the environmental conditions provided have been simple—space, oxygen, water exchange, and occasionally spawning mats. We would like to retain the idea (proposed in a previous paper, Sneed and Clemens, 1960) that any physical or chemical stimuli which normally cause the fish's own pituitary to secrete the gonadotropins necessary to precipitate the spawning act were bypassed, so to speak, by the exogenous gonadotropins. However, the successful spawning of two pairs of rock bass included some environmental additions which at this point seem necessary.

Two pairs of rock bass were spawned under similar conditions (table 12). A water temperature of 76° F., a coarse gravel bottom with a slight current of water flowing over the gravel, and a five-gallon aquarium constituted the facilities. Both males and females received two injections of buffalo pituitary 24 hours apart, amounting to a dosage of about 2 milligrams. Eggs were scattered over the gravel and were adhesive. They hatched in 3 days and were reared successfully.

Other experiments involved gravel and aeration (without running water), no gravel and running water, no gravel and aeration; each failed to induce spawning. However, two additional, unsuccessful attempts were made in what was considered to be favorable conditions. In these experiments, as well as in the other unsuccessful ones, the condition (gonadal development) of the fish may have been a limiting factor.

Zebra fish (*Brachydanio rerio*)

After a great deal of preliminary experimentation with zebra fish, a larger experiment was attempted. One hundred adult zebras were bought from a tropical fish supply house. Twenty-six females were selected, and 24 of these were injected with 0.6 milligrams of acetone-dried carp pituitaries in 0.2 milliliters of distilled water. The remaining 74 fish were either males or fish difficult to sex. Ten injected and two control females were placed in individual spawning containers (battery jars), with plastic screen suspended two inches from the bottom to protect the eggs. Fourteen injected females were placed together in an aquarium.[1] Two fish believed to be males were stocked per female; i.e., 28 such fish were used. None of the males were injected. Injections were made at 6 p.m., and four females died shortly after being injected. The following morning, four of the six living females in individual containers had spawned a total of 340 eggs (table 14), while one of the uninjected females had spawned. The spawning of one of the control females considerably weakens, in this instance, the case for inducing zebra fish to spawn. However, since this species readily spawns in small containers, the difference in injected fish over the controls will never be as clear cut as in fish with more precise spawning requirements, such as channel catfish, rock bass, etc. The egg count of the females that spawned individually, including the two which did

TABLE 14.—*Results of injecting zebra fish with 0.6 milligram of acetone-dried carp pituitary in 0.2 milliliter of distilled water*

Number of females	Number of males per female	Number of ovulating females	Number of fertile eggs	Total number of eggs	Number of females dead	Percent of fertile eggs
Selected brood stock:						
1	2	1	93	112		83.1
1	2	1	0	1		0
1	2	0	0	2	[1] 5	0
1	2	0	33	65		50.7
1	2	1	121	143		84.6
1	1	0	11	17		64.7
1 (control)	2	0	0	0		0
1 (control)	2	0	50	52		96.1
14	1	4	272	366		74.3
Culls: [2]						
33			116	121	14	95.8

[1] Four of these fish died shortly after injection and they were not included in the remainder of the data.
[2] These fish were not sexed.

not spawn, averaged 57 eggs per female with a range from 17 to 143 eggs. On the basis of this average, about 6 of the 14 females spawned in the aquarium experiment.

The culls from this lot of fish were injected with the same amount of pituitaries of the same pituitary lot. In this experiment both males and females were injected because the poorly developed females and the more immature fish were difficult to sex. Eleven of 33 fish died immediately after injection, 3 died the following day, and several others were rather sluggish. The fish first lost their equilibrium and their ability to swim; their scales began to protrude; and in the final stages, the body became rigid. From the remaining 22 injected fish, 121 eggs were obtained, a yield of 11 eggs per female if a 1:1 sex ratio existed.

The percent of fertile eggs found in each spawning container varied from 51 to 96, the highest being in the uninjected control fish and in the culls. There are a number of possible reasons for the lowered fertility: (1) the exogenous gonadotropin might have had a deleterious effect on the eggs; (2) the amount of gonadotropin injected might have been excessively high and caused the female to void her eggs too rapidly and they settled through the screen before the male could fertilize them; (3) the males were in poor condition and incapable of fertilizing many eggs; (4) the number of males required to spawn a female and fertilize the eggs was inadequate.

The fact that the percentage of fertility in the eggs from the cull fish approximates that in the controls suggests that exogenous gonadotropins do not necessarily lower the fertility and that the lower fertility in the eggs of the select fish was perhaps due to an inadequate number of males. It may be wise to stock males and females in ratios greater than 1:1 or 2:1, and place the plastic netting near the bottom to insure proximity of the eggs and sperm.

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